International Journal of Nutrology
(Official Journal of the ABRAN - Brazilian Association of Nutrology)

REVIEW ARTICLE

DOI: 10.54448/ijn22309

Major approaches to melatonin and nutrients regulation in the bone regeneration process with exosomes and microRNAs: a systematic review

Danillo Gomes Leite[1]*

[1] Sports Clinic and Hospital Israelita Albert Einstein, Goiânia, Goiás, Brazil.

Corresponding Author: Dr. Danillo Gomes Leite. Sports Clinic and Hospital Israelita Albert Einstein, Goiânia, Goiás, Brazil.
E-mail: danillogl@hotmail.com
DOI: https://doi.org/10.54448/ijn22309
Received: 05-25-2022; Revised: 09-15-2022; Accepted: 10-02-2022; Published: 10-10-2022; IJN-id: e22309

Abstract

Introduction: Bone diseases comprise a large group of common diseases, including fractures, osteoporosis, and osteoarthritis that affect a large number of individuals. Without intervention, the prevalence of osteopenia is projected to increase to 64.3 million Americans and that of osteoporosis to 11.9 million by the year 2030. Melatonin exerts numerous physiological effects, including the induction of anti-inflammatory and antioxidants, resetting circadian rhythms, and promoting wound healing and tissue regeneration, participating in the maintenance and regenerative processes of bones and cartilage. **Objective:** A systematic review was carried out to present the state of the art of melatonin regulation, mesenchymal stem cells, exosomes, microRNAs, and nutrients in the bone regeneration process. **Methods:** The systematic review rules (PRISMA) were followed. The search was carried out from July to September 2022 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases, using scientific articles from 2019 to 2022. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** A total of 126 articles were found. A total of 59 articles were fully evaluated and 46 were included in this systematic review. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 9 studies at high risk of bias and 24 studies that did not meet the GRADE. Most studies showed homogeneity in their results, with I2 =97.8%>50%. The symmetrical funnel plot does not suggest a risk of bias between small sample-size studies. Based on the results, melatonin has important functions in regulating the regenerative activities of mesenchymal stem cells that modulate, together with nutrients, the activities of exosomes and microRNAs in the bone regeneration process.

Keywords: Bone diseases. Bone regeneration. Cartilage regeneration. Melatonin. Nutrients. Exosomes. MicroRNAs.

Introduction

Bone diseases comprise a large group of common diseases, including fractures, osteoporosis, and osteoarthritis that affect a large number of individuals, particularly the elderly. Without intervention, the prevalence of osteopenia is projected to increase to 64.3 million Americans and that of osteoporosis to 11.9 million by the year 2030 **[1]**.

With existing prevention and treatment methods, the incidence and mortality of bone diseases are still steadily increasing, creating a significant financial burden for societies across the world. To prevent the occurrence of bone diseases, slow their progression, or reverse the injuries they cause, new alternatives or complementary treatments need to be developed. Thus, melatonin exerts numerous physiological effects, including inducing anti-inflammatory and antioxidant functions, resetting circadian rhythms, and promoting wound healing and tissue regeneration. Melatonin also participates in the maintenance and regenerative processes of bones and cartilage **[2]**.

In this context, research has advanced on the physiological role of melatonin (MEL) and its pharmacological analogs as therapeutic agents for the treatment of various pathologies. Thus, in the last 20 years, solid experimental and some clinical evidence has accumulated on the important role of MEL in the regulation of metabolism **[3,4]**.

The sleep-wake cycle is critical for the secretion and physiological variations of several hormones,

including MEL [5]. Indolaminergic melatonin (N-acetyl-5-methoxytryptamide) is a hormone produced mainly by the pineal gland, but also in the gastrointestinal tract, retina, lacrimal glands, skin, erythrocytes, platelets, lymphocytes, and bone marrow mononuclear cells, derived from noradrenergic stimulation. of tryptophan and serotonin by α1 and β1 adrenoceptors on postsynaptic pinealocytes [6].

Unlike other hormonal axes, MEL secretion is not regulated by feedback and, therefore, its plasma concentrations do not depend on its production. The pineal gland secretion has its control influenced by the circadian cycle in the suprachiasmatic nucleus of the hypothalamus and, consequently, promotes the peak of MEL secretion during the night, and during the day it decreases by exposure to light [7].

In addition, MEL has both endocrine and paracrine actions and binds to three receptors, central and peripheral, at various sites in the body [8]. The high-affinity receptors MT1 and MT2 or MTNR1A and MTNR1B belong to the family of membrane-bound receptors with G protein activation by PKC and cyclic GMP-reduced monophosphate (cGMP), respectively. MT3, a recently discovered nuclear receptor of the retinoic acid family (RZR/ROR), has a quinone reductase-like structure whose function is not yet fully understood [9].

In this sense, there is a decrease in MEL secretion with aging and the presence of various diseases [9]. The sleep pattern changes and this has a great impact with advancing age and the development of certain diseases such as osteoporosis and osteoarthritis [10].

Associated with the effects of MEL, stem cells from adult tissue (mesenchymal stem cells) mediate homeostasis and regeneration of tissues and organs, making decisions about whether to remain quiescent, proliferate, or differentiate into mature cell types. These decisions are directly integrated with the body's energy balance and nutritional status. By-products and metabolic substrates that regulate epigenetic and signaling pathways are considered to have an instructive role, rather than an observer, in the regulation of cell fate decisions [11].

In this sense, it is suspected that the quiescent state of stem cells is characterized by an inherently glycolytic metabolism, followed by a transition to favor mitochondrial oxidative phosphorylation during differentiation [12-15]. However, increasing evidence suggests that metabolism during quiescence, activation, and differentiation may vary between tissues, integrating signaling cues and metabolic inputs with the release of exosomes and microRNAs as important metabolic messengers in the body, this process is strongly regulated by nutrients.

In this scenario, nutrient-mediated metabolomics provides information on cellular pathways, observing substrates and metabolic products through different pathways [16,17]. Along with transcriptomics and proteomics analysis, it is observed that metabolism can affect cell fate (and vice versa) [18].

Therefore, the present study aimed to carry out a systematic review to present the state of the art of melatonin regulation, mesenchymal stem cells, exosomes, microRNAs, and nutrients in the bone regeneration process.

Methods

Study Design

The present study followed a concise systematic review model, following the rules of systematic review - PRISMA (Transparent reporting of systematic review and meta-analysis-HTTP: //www.prisma-statement.org/).

Search Strategy and Search Sources

The literary search process was carried out from July to September 2022 and was developed based on Scopus, PubMed, Science Direct, Scielo, and Google Scholar, using scientific articles from 2019 to 2022, using the descriptors (MeSH Terms): Melatonin, and using the Booleans "and" between the MeSH terms and "or" between the historical findings.

Study Quality and Risk of Bias

Quality was rated as high, moderate, low, or very low for risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident highlight was for systematic review articles or meta-analysis of randomized clinical trials, followed by randomized clinical trials. The low quality of evidence was attributed to case reports, editorials, and brief communications, according to the GRADE instrument. The risk of bias was analyzed according to the Cochrane instrument through the analysis of the Funnel Plot (Sample Size versus Effect Size), using the Cohen test (d).

Results and discussion of the systematic review

Summary of Findings

As a corollary of the literary search system, 126 studies were analyzed and submitted to eligibility analysis, and then 46 of the 59 final studies were selected for this systematic review. The listed studies presented medium to high quality (**Figure 1**), considering in the first instance the level of scientific

evidence of studies in study types such as meta-analysis, consensus, randomized clinical, prospective and observational. The biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies showed homogeneity in their results, with I2 =97.8% >50%.

Figure 1. Flowchart showing the article selection process.

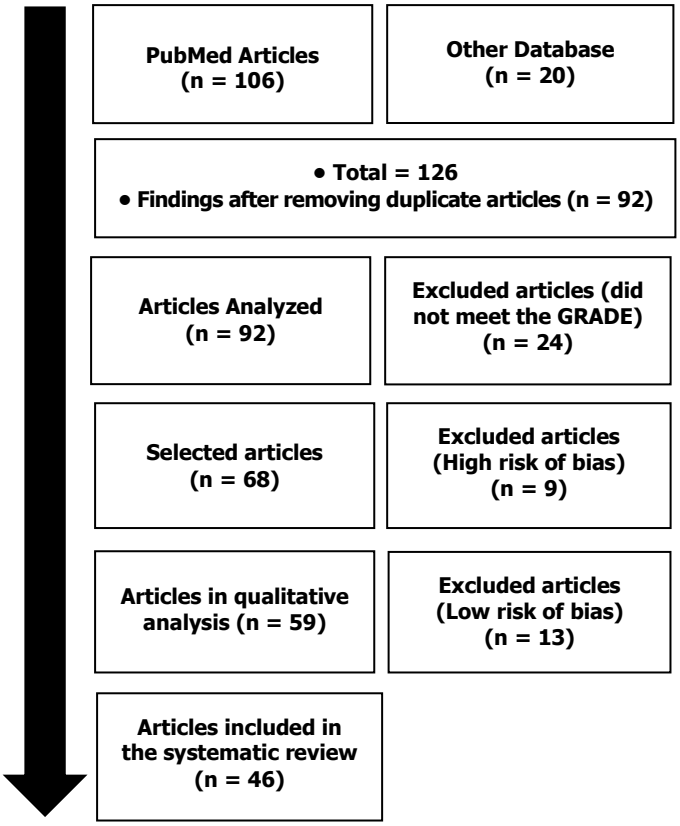
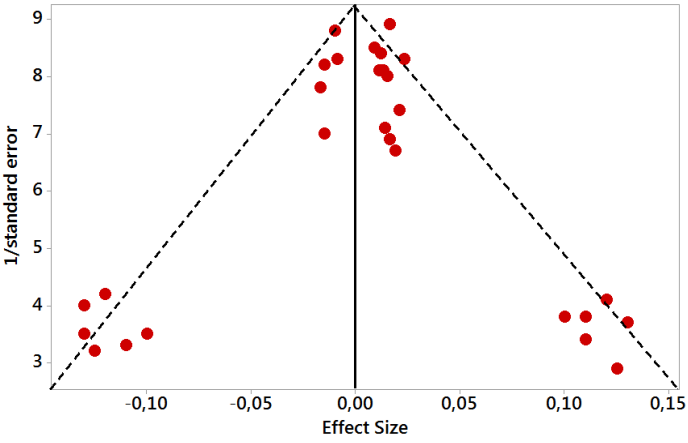


Figure 2 presents the results of the risk of bias of the studies using the Funnel Plot, showing the calculation of the Effect Size (Magnitude of the difference) using the Cohen Test (d). Precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph had a symmetrical behavior, not suggesting a significant risk of bias, both between studies with a small sample size (lower precision) that are shown at the bottom of the graph and in studies with a large sample size that are presented in the upper region.

Melatonin - Metabolomics and Bone/Chondrogenic Regeneration

In the endocrine physiology scenario, due to its characteristic of an amphiphilic molecule, MEL can cross cells, organelles, and nuclear membranes and directly interact with intracellular molecules in the so-called non-receptor-mediated actions **[3]**.

Figure 2. The symmetrical funnel plot does not suggest a risk of bias between the small sample size studies that are shown at the bottom of the plot. High confidence and high recommendation studies are shown above the graph (n=46 clinical studies).



MEL is a well-known effective antioxidant, as it is both a proficient scavenger of direct free radicals and an activator of some scavenging mechanisms, such as a stimulator of transcription and activity of antioxidant enzymes, and it binds to transition metals that inhibit the formation of hydroxyls. In addition, MEL protects lipids, proteins, and DNA against oxidative damage and is highly concentrated in mitochondria **[4]**.

In this context, the antioxidant properties of MEL are of crucial importance for mitochondrial functions, playing critical roles in mitochondrial function in addition to antioxidant protection such as regulating the activities of respiratory complexes I and IV and protecting mitochondrial DNA against chromosomal/chromatid alterations and mutations **[5]**. Thus, some of the aforementioned effects are generally a consequence of direct MEL-protein interaction. It is also notable that MEL plays a role in regulating the ubiquitin-proteasome system that ultimately controls protein degradation **[6]**.

Furthermore, MEL has been reported to inhibit Ca2+/calmodulin which is dependent on protein kinase II activity and autophosphorylation by direct interaction with Ca2+-activated calmodulin, acting as an antagonist. It has also been suggested that MEL influences the expression of circadian rhythm genes **[19]**.

The MEL MT1 and MT2 receptors formerly termed MEL1a and MEL1b are specific high-affinity G protein-coupled receptors encoded by MTNR1A and MTNR1B genes, which have been found in several areas of the CNS, including the CNS, midbasal hypothalamus, thalamus, temporal, parietal and frontal cortex, hippocampus, preoptic basal ganglia, area postrema,

retina, cerebellum, and pars tuberalis region, as well as adipose tissue, kidney, pancreas, islets, parotid glands, adrenal glands, liver, bone, skin, reproductive tract, immune cells, and cardiovascular system **[20]**.

In this sense, MEL MT1 and MT2 receptors are heterotrimeric Gi/Go and Gq protein-coupled receptors that interact with messengers such as adenylyl cyclase, phospholipase A, phospholipase C, and calcium potassium channels, generally decreasing cAMP and cGMP and/or cGMP production. or activation of phospholipase C. Thus, MT1 and MT2 generally dimerize, forming homodimers or heterodimers that maintain the two MEL binding sites functional and with their respective selectivity **[20]**.

Furthermore, GPR61/62 and GPR135 are other G protein-coupled receptors that can dimerize for MT Reducing, reducing its affinity with MEL and its agonists, being a potential regulatory step in a signaling mechanism. MT signaling pathways involve, for example, the activation of potassium K ion channels that mediate inhibition of neuronal firing in the SCN. Modulation of protein kinase C (PKC) and phospholipase A1 **[21]**.

Furthermore, MT3 is a third binding site of mammalian MEL which is a form of quinone reductase, a detoxifying enzyme, and has been reported to be involved in the enhancement of chemotherapy-induced and MEL-apoptosis-derived cytotoxicity in tumor cell lines. Furthermore, MEL can also interact with nuclear receptors of the retinoic acid-related receptor (ROR), retinoid Z receptor group **[22]**.

Despite all these findings from the physiological functions of MEL, the metabolic pathways involved in human sleep still need to be investigated using a metabolomics approach. Thus, one study performed targeted liquid chromatography (LC)/MS metabolomics to examine the effect of acute sleep deprivation on plasma metabolite rhythms. Twelve healthy young male subjects remained under controlled laboratory conditions regarding ambient light, sleep, meals, and posture during a 24-hour wake/sleep cycle followed by 24 hours of wakefulness. Two-hour plasma samples collected during the 48 hours were analyzed by LC/MS. Principal component analysis revealed a clear variation of the time of day with a significant cosine adjustment during the wake/sleep cycle and during 24 hours of wakefulness in undirected and directed analyses. Of the 171 quantified metabolites, daily rhythms were observed in the majority (n = 109), with 78 of them maintaining their rhythm during 24 hours of wakefulness, most with reduced amplitude (n = 66). During sleep deprivation, 27 metabolites (tryptophan, serotonin, taurine, 8 acylcarnitines, 13 glycerophospholipids, and 3 sphingolipids) exhibited significantly increased levels compared to sleep. The increased levels of serotonin, tryptophan, and taurine may explain the antidepressant effect of acute sleep deprivation **[19]**.

In this context, MEL is considered a potent cytoprotective agent, not just a hormone **[21,22]**. MEL can synchronize the circadian clock in peripheral tissues, maintain the synchronization of bone metabolism with the Light/Dark cycles and participate in numerous important physiological processes, such as anti-inflammatory, antitumor, and antioxidant processes, in addition to regulating circadian and endocrine rhythms, regulating immunity and promoting wound healing and tissue regeneration **[23,24]**.

Furthermore, MEL plays an important role in bone-related diseases. While there are many physical and drug treatments for bone diseases, MEL has the advantage over other drugs that are low cost, wide margin of safety, have wide tissue impact, and have almost no side effects, suggesting its potential as a main or complementary treatment strategy for a wide variety of bone diseases **[24]**.

In this aspect, MEL is involved in the regulation of bone mass accumulation and loss. Egermann et al **[25]** confirmed that bone mass significantly decreases after pinealectomy. Decreased MEL secretion is associated with menopause and is one of the most important causes of osteoporosis **[26]**. The production of MEL decreases with age, which can lead to greater bone loss among the elderly **[27]**. Furthermore, the expression of melatonin receptor 1A (MTNR1A) on the surface of human osteoblasts decreases with age, with a higher frequency in women **[28]**.

Exogenous melatonin supplementation is effective and safe, bringing more osteoblasts and fewer osteoclasts. The application of melatonin can reduce high levels of the NLRP3 inflammasome in individuals suffering from estrogen deficiency. Melatonin also attenuates osteoblast autophagy in patients with diabetes mellitus, which is considered beneficial in reducing bone loss. In addition, melatonin regulates calcium metabolism and prevents osteoporosis **[28]**.

Furthermore, inflammatory processes play a crucial role in the pathogenesis of osteoarthritis (OA), as mild and chronic inflammation has been shown to contribute to the symptoms and progression of OA **[29,30]**. The ability of cartilage to self-repair is limited, with the ability to repair cell-based articular cartilage in inflamed joints being even lower. Thus, melatonin intervention may partially restore the chondrogenic differentiation capacity of mesenchymal stem cells affected by IL-1β-induced inflammation **[31,32]**. The

effect of the long-term intervention (21 days) is significant. Melatonin can also reduce the phosphorylation of p65 and IκBα, thus inhibiting the activation of the downstream NF-κB signaling pathway, which plays a key role in metabolism, inflammation, and apoptosis **[33]**.

Also, multiple microRNAs (miRNAs/miRs) are involved in OA **[34]**. As an example, miR-140-5p is expressed in cartilage and plays an important role in chondrocyte differentiation and cartilage degeneration **[35]**. OA-associated cartilage changes occur in mice lacking miR-140 **[36]**, while overexpression of miR-140 has been shown to inhibit matrix catabolic enzyme synthesis **[37]**. Elevated levels of pro-inflammatory cytokines in cartilage can reduce miR-140 expression **[38]**.

In this sense, MEL plays a protective role in OA-induced cartilage degradation by upregulating miR-140 and activating SMAD signaling pathways **[32]**, which can inhibit NF-κB pathways in articular cartilage **[39]**. In addition, other miRNAs that participate in cartilage protection, such as miR-526b-3p and miR-590-5p, can be upregulated by melatonin, improving the chondrogenic differentiation of mesenchymal stem cells **[40]**.

Main Cellular and Molecular Processes of Bone Regeneration

In this scenario, adult stem cells, such as mesenchymal stem cells (MSC), point to an alternative for cell therapy and human tissue engineering, since it was found that they have a high degree of plasticity, with the ability to self-regenerate-renewal and differentiation into specialized progenitors **[41]**.

In this aspect, MSCs are primordial mesodermal cells present in all tissues and are capable of differentiating in vitro and in vivo into different cell types. Its therapeutic potential is mainly explained by the production of bioactive molecules, which provide a regenerative microenvironment in injured tissues **[42]**. Furthermore, MSCs secrete a cascade of cytokines and growth factors with paracrine, autocrine, and endocrine activities, such as Il-6, Il-7, Il-8, Il-11, Il-12, Il-14, Il-15, macrophage colony-stimulating factor (M-CSF), Flt-3 ligand and Stem Cell Factor (SCF), leukemia inhibitory factor (LIF), granulocytic colony-stimulating factor (G-CSF) and granulocytic colony-stimulating factor - macrophages (GM-CSF). These factors when conjugated can produce a series of responses in the local immune system, stimulating angiogenesis and inducing the proliferation and differentiation of mesenchymal stem cells in the desired tissue **[43]**.

In addition, MSCs induce the expression of junction proteins and increase microvascular integrity and the production of nitric oxide (NO) by macrophages **[42]**. The vascular stromal fraction (VSF) from MSCs is a heterogeneous mixture of cells, including fibroblasts, pericytes, endothelial cells, blood cells, and mesenchymal stem cells derived from adipose tissue (AMSC).

In addition, exosomes stand out together with AMSC. Exosomes are extracellular vesicles with a size of 40-100 nm in diameter and a density of 1.13-1.19 g/mL, containing proteins, mRNAs, miRNAs, and DNAs. Exosomes change the biochemical characteristics of recipient cells through the delivery of biomolecules and play a role in cellular communication. These vesicles are produced from body fluids and different types of cells. Evidence suggests that the AMSC-derived exosome (AMSC -EXO) exhibits AMSC -like functions with low immunogenicity and no tumorization **[44]**.

In this sense, the composition of exosomes differs based on their sources. The protein and lipid content of exosomes was measured by various methods, such as fluorescence-activated cell selection, Western blotting, mass spectrometry, and immunoelectron microscopy. In this regard, Rabs and Annexin, including Annexin I, II, V, and VI are cytosolic proteins present in exosomes that contribute to the formation of exosome docking, membrane fusion, and kinetic regulation of cytoskeletal membranes. Furthermore, adhesion molecules such as intercellular adhesion molecule-1, CD11a, CD11b, CD11c, CD18, CD9 adipose tissue globule-EGF-factor VIII (MFG-E8), CD58, CD146, CD166 have also been identified on exosomes **[45]**. Exosomes also contain heat shock proteins (Hsp70 and Hsp90), which facilitate the loading of peptides into MHC I and II **[46,47]**.

Besides, exosomes contain RNAs or non-coding fragments, including overlapping RNA transcripts, protein-coding region, structural RNAs, transfer RNA fragments, YRNAs, short hairpin RNAs, small interfering RNAs (siRNAs), microRNA (miRNA), messenger RNA (mRNA) and DNA **[48]**. Regarding miRNA, exosomes present miR-1, miR-15, miR-16, miR-17, miR-18, miR-181 and miR-375 **[49]**. In addition, various cytokines such as Tumor Necrosis Factor-α (TNF-α), Granulocyte Macrophage Colony Stimulating Factor (GMCSF), Interleukin (IL)-2, IL-6, IL-8, IL-10, IL-15, IL-1β, are expressed in exosomes **[50]**.

Based on this, normal bone formation and tissue repair involve coordinated interaction between bone-forming cells and biological signals. The main force in this process is osteoblasts and their precursors **[51]**. Osteoblasts can produce new bone along with biomaterials and can initiate the release of biological

signals that guide bone formation and remodeling.

These biological signals attract bone-forming cells to the receptor site. Growth factors and other proteins are some of the biological signals that may be involved in bone neoformation and tissue remodeling. In addition, through chemotaxis, there is a migration of bone-forming cells to the application area, as the stimulation of cell migration occurs in response to chemical stimuli **[52]**.

In this sense, monocytes, macrophages, and endothelial cells contribute to bone remodeling, either through contact with osteogenic cells or through the release of soluble factors such as cytokines and GF **[52]**. In the skeletal system, TNF-α stimulates bone and cartilage resorption and inhibits collagen and proteoglycan synthesis. IL-1 induces the expression of a wide variety of cytokines. LIF and IL-6 are two such molecules that are known to stimulate the differentiation of mesenchymal progenitor cells in the osteoblastic lineage, they are also potent anti-apoptotic agents for osteoblasts. In bone, the main sources of IL-6 are osteoblasts and not osteoclasts. Prostaglandin E2 (PGE2) is also directly related to the expression of the cytokine IL-6 **[53,54]**.

A study by Liang et al. 2022 **[55]** showed that exosomes derived from mesenchymal stem cells (MSC-Exos) perform the regulatory function of stem cells transporting proteins, nucleic acids, and lipids. Intervertebral disc degeneration (IDD) is one of the main causes of low back pain and is characterized by a decrease in the number of cells in the nucleus pulposus, decomposition of the extracellular matrix, aging of the annulus fibrosus and calcification of the cartilage endplate. Furthermore, nutrient transport and structural repair of intervertebral discs depend on bone and cartilage and are closely related to the state of the bone. Trauma, illness, and aging can all cause bone damage. Recent fine-tuning of the CTM-Exos has led to significant progress in the treatment of DDI and bone repair and regeneration.

Regenerative Processes and Nutrology

In the context of regenerative processes, endogenous metabolites and dietary nutrients can directly influence epigenetic enzymes. Epigenetic modifications in DNA and histone proteins alter the fate of the cell by controlling chromatin accessibility and downstream gene expression patterns **[56]**.

In this sense, many substrates and cofactors for chromatin-modifying enzymes are derived from metabolic pathways involving the tricarboxylic acid cycle, the methionine cycle, the folate cycle, glycolysis, β-oxidation, and the hexosamine pathway. These metabolites can serve as activators or inhibitors of epigenetic writers, such as proteins containing the Jumonji C domain (JmjC), DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), ten-eleven DNA translocase demethylases (TETs) and histone deacetylases (HDACs). In this sense, metabolites can influence nutrient detection signaling pathways **[56]**.

Thus, the mechanistic target of the rapamycin complex 1 (mTORC1) can be activated by growth factor-induced signaling only when the amino acids arginine and leucine, as well as the cofactor S-adenosyl methionine (SAM), are detected within the cell. Furthermore, the energy balance communicated through the cellular AMP/ADP-ATP ratio can be detected by AMP-activated protein kinase (AMPK). In addition, transcription factors can be directly regulated by metabolites, for example, the tryptophan kynurenine metabolite is an endogenous agonist of the aryl hydrocarbon receptor and alpha-ketoglutarate (α-KG) binds and activates IKKβ and initiates IKKβ signaling. NF-κβ **[56]**.

Also, epigenetic signaling pathways and transcription are affected by changing nutrient levels. Furthermore, the focus of the literature on stem cell metabolism is centered on central carbon metabolism and the balance between glycolysis and oxidative phosphorylation in the regulation of cell fate **[57]**. Therefore, future research that defines the dietary and metabolic control of decisions about the fate of cells in muscle tissues will be of great importance in the fields of metabolism and regenerative medicine.

Over the past decade, several flavonoids have been reported to have osteogenic-angiogenic potential in bone regeneration due to their excellent bioactivity, low cost, availability, and minimal in vivo toxicity. During new bone formation, the osteoinductive nature of certain flavonoids is involved in the regulation of multiple signaling pathways that contribute to osteogenic-angiogenic coupling **[58]**.

Besides, a meta-analysis study identified micronutrients from the "European Union (EU) Register of Nutrition and Health Claims Made on Foods" that are related to bone health. 19 studies were identified that demonstrated the importance of vitamin D, magnesium, resveratrol, vitamin C, a mixture of calcium, magnesium, zinc, and vitamin D, and synthetic bone mineral in the processes of bone formation and maintenance **[59]**.

Conclusion

It was concluded that melatonin has important functions in the regulation of regenerative activities of

mesenchymal stem cells that modulate, together with nutrients, the activities of exosomes and microRNAs in the bone regeneration process.

Acknowledgement
Not applicable.

Ethics approval
Not applicable.

Informed consent
Not applicable.

Funding
Not applicable.

Data sharing statement
No additional data are available.

Conflict of interest
The authors declare no conflict of interest.

References

1. Maria S, Witt-Enderby PA. Melatonin effects on bone: Potential use for the prevention and treatment for osteopenia, osteoporosis, and periodontal disease and for use in bone-grafting procedures. J Pineal Res. 2014;56:115–125. doi: 10.1111/jpi.12116.
2. Lu X, Yu S, Chen G, Zheng W, Peng J, Huang X, Chen L. Insight into the roles of melatonin in bone tissue and bone related diseases (Review). Int J Mol Med. 2021 May;47(5):82. doi: 10.3892/ijmm.2021.4915. Epub 2021 Mar 24. PMID: 33760138; PMCID: PMC7979260.
3. Pivonello C, Negri M, Patalano R, Amatrudo F, Montò T, Liccardi A, Graziadio C, Muscogiuri G, Pivonello R, Colao A. The role of melatonin in the molecular mechanisms underlying metaflammation and infections in obesity: A narrative review. Obes Rev. 2022 Mar;23(3):e13390. doi: 10.1111/obr.13390. Epub 2021 Dec 3. PMID: 34861097; PMCID: PMC9285339.
4. Boga JA, Caballero B, Potes Y, Perez-Martinez Z, Reiter RJ, Vega-Naredo I, Coto-Montes A. Therapeutic potential of melatonin related to its role as an autophagy regulator: A review. J Pineal Res. 2019 Jan;66(1):e12534. doi: 10.1111/jpi.12534. Epub 2018 Nov 26.
5. Guan Q, Wang Z, Cao J, Dong Y, Chen Y. Mechanisms of Melatonin in Obesity: A Review. Int J Mol Sci. 2021 Dec 25;23(1):218. doi: 10.3390/ijms23010218.
6. Delpino FM, Figueiredo LM. Melatonin supplementation and anthropometric indicators of obesity: A systematic review and meta-analysis. Nutrition. 2021 Nov-Dec;91-92:111399. doi: 10.1016/j.nut.2021.111399. Epub 2021 Jun 24. PMID: 34626955.
7. Baron KG, Reid KJ, Wolfe LF, Attarian H, Zee PC. Phase Relationship between DLMO and Sleep Onset and the Risk of Metabolic Disease among Normal Weight and Overweight/Obese Adults. J Biol Rhythms. 2018 Feb;33(1):76-83. doi: 10.1177/0748730417745914.
8. Cardinali DP, Vigo DE. Melatonin, mitochondria, and the metabolic syndrome. Cell Mol Life Sci. 2017 Nov;74(21):3941-3954. doi: 10.1007/s00018-017-2611-0. Epub 2017 Aug 17.
9. Rao PV. Type 2 diabetes in children: clinical aspects and risk factors. Indian J Endocrinol Metab 2015; 19(Suppl1): S47-S50.
10. Milcu I, Nanu L, Marcean R et al. The action of pineal extract and epiphysectomy on hepatic and muscular glycogen after prolonged infusion of glucose. Stud Cercet Endocrinol 1963; 14: 651-655.
11. Chacón-Martínez CA et al. (2017) Hair follicle stem cell cultures reveal self-organizing plasticity of stem cells and their progeny. EMBO J. 36, 151–164.
12. Rodríguez-Colman, M.J. et al. (2017) Interplay between metabolic identities in the intestinal crypt supports stem cell function. Nature 543, 424.
13. Snoeck, H.W. (2017) Mitochondrial regulation of hematopoietic stem cells. Curr. Opin. Cell Biol. 49, 91–98.
14. Zheng, X. et al. (2016) Metabolic reprogramming during neuronal differentiation from aerobic glycolysis to neuronal oxidative phosphorylation. Elife 5, e13374.
15. Flores, A. et al. (2017) Lactate dehydrogenase activity drives hair follicle stem cell activation. Nat. Cell Biol. 19, 1017–1026.

16. Rinschen MM. et al. (2019) Identification of bioactive metabolites using activity metabolomics. Nat. Rev. Mol. Cell Biol. 20, 353–367.
17. Agathocleous, M. et al. (2017) Ascorbate regulates haematopoietic stem cell function and leukaemogenesis. Nature 549, 476–481.
18. Shapira SN, Christofk HR. Metabolic Regulation of Tissue Stem Cells. Trends Cell Biol. 2020 Jul;30(7):566-576. doi: 10.1016/j.tcb.2020.04.004. Epub 2020 Apr 28. PMID: 32359707.
19. Davies SK, Ang JE, Revell VL, Holmes B, Mann A, Robertson FP, Cui N, Middleton B, Ackermann K, Kayser M, Thumser AE, Raynaud FI, Skene DJ. Effect of sleep deprivation on the human metabolome. Proc Natl Acad Sci U S A. 2014 Jul 22;111(29):10761-6. doi: 10.1073/pnas.1402663111. Epub 2014 Jul 7.
20. Al-Sarraf IAK, Kasabri V, Akour A, Naffa R. Melatonin and cryptochrome 2 in metabolic syndrome patients with or without diabetes: a cross-sectional study. Horm Mol Biol Clin Investig. 2018 May 29;35(2). pii: /j/hmbci.2018.35.issue-2/hmbci-2018-0016/hmbci-2018-0016.xml. doi: 10.1515/hmbci-2018-0016.
21. Tan DX, Manchester LC, Hardeland R, Lopez-Burillo S, Mayo JC, Sainz RM, Reiter RJ. Melatonin: A hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J Pineal Res. 2003;34:75–78. doi: 10.1034/j.1600-079X.2003.02111.x.
22. Permuy M, López-Peña M, González-Cantalapiedra A, Muñoz F. Melatonin: A review of its potential functions and effects on dental diseases. Int J Mol Sci. 2017;18:865. doi: 10.3390/ijms18040865.
23. Amaral FGD, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. Arch Endocrinol Metab. 2018;62:472–479. doi: 10.20945/2359-3997000000066.
24. Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, Fougerou C. Melatonin: Pharmacology, functions and therapeutic benefits. Curr Neuropharmacol. 2017;15:434–443. doi: 10.2174/1570159X14666161228122115.
25. Egermann M, Gerhardt C, Barth A, Maestroni GJ, Schneider E, Alini M. Pinealectomy affects bone mineral density and structure-an experimental study in sheep. BMC Musculoskelet Disord. 2011;12:271. doi: 10.1186/1471-2474-12-271.
26. Pines A. Circadian rhythm and menopause. Climacteric. 2016;19:551–552. doi: 10.1080/13697137.2016.1226608.
27. Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM. Human melatonin production decreases with age. J Pineal Res. 1986;3:379–388. doi: 10.1111/j.1600-079X.1986.tb00760.x.
28. Satomura K, Tobiume S, Tokuyama R, Yamasaki Y, Kudoh K, Maeda E, Nagayama M. Melatonin at pharmacological doses enhances human osteoblastic differentiation in vitro and promotes mouse cortical bone formation in vivo. J Pineal Res. 2007;42:231–239. doi: 10.1111/j.1600-079X.2006.00410.x.
29. Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, Sokolove J. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol. 2016;12:580–592. doi: 10.1038/nrrheum.2016.136.
30. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. Nat Rev Rheumatol. 2015;11:35–44. doi: 10.1038/nrrheum.2014.162.
31. Gao B, Gao W, Wu Z, Zhou T, Qiu X, Wang X, Lian C, Peng Y, Liang A, Qiu J, et al. Melatonin rescued interleukin 1β-impaired chondrogenesis of human mesenchymal stem cells. Stem Cell Res Ther. 2018;9:162. doi: 10.1186/s13287-018-0892-3.
32. Zhang Y, Lin J, Zhou X, Chen X, Chen AC, Pi B, Pan G, Pei M, Yang H, Liu T, He F. Melatonin prevents osteoarthritis-induced cartilage degradation via targeting MicroRNA-140. Oxid Med Cell Longev. 2019;2019:9705929. doi: 10.1155/2019/9705929.
33. Hosseinzadeh A, Kamrava SK, Joghataei MT, Darabi R, Shakeri-Zadeh A, Shahriari M, Reiter RJ, Ghaznavi H, Mehrzadi S. Apoptosis signaling pathways in osteoarthritis and possible protective role of melatonin. J Pineal Res. 2016;61:411–425. doi: 10.1111/jpi.12362.
34. Nugent M. MicroRNAs: Exploring new horizons in osteoarthritis. Osteoarthritis Cartilage. 2016;24:573–580. doi: 10.1016/j.joca.2015.10.018. [PubMed] [CrossRef] [Google Scholar]
35. Miyaki S, Asahara H. Macro view of microRNA function in osteoarthritis. Nat Rev Rheumatol. 2012;8:543–552. doi: 10.1038/nrrheum.2012.128.

36. Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, Kato Y, Takemoto F, Nakasa T, Yamashita S, et al. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev. 2010;24:1173–1185. doi: 10.1101/gad.1915510.

37. Si HB, Zeng Y, Liu SY, Zhou ZK, Chen YN, Cheng JQ, Lu YR, Shen B. Intra-articular injection of microRNA-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. Osteoarthritis Cartilage. 2017;25:1698–1707. doi: 10.1016/j.joca.2017.06.002.

38. Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R, Inoue A, Kato Y, Sato T, Lotz MK, Asahara H. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum. 2009;60:2723–2730. doi: 10.1002/art.24745.

39. Karlsen TA, de Souza GA, Ødegaard B, Engebretsen L, Brinchmann JE. microRNA-140 inhibits inflammation and stimulates chondrogenesis in a model of interleukin 1β-induced osteoarthritis. Mol Ther Nucleic Acids. 2016;5:e373. doi: 10.1038/mtna.2016.64.

40. Wu Z, Qiu X, Gao B, Lian C, Peng Y, Liang A, Xu C, Gao W, Zhang L, Su P, et al. Melatonin-mediated miR-526b-3p and miR-590-5p upregulation promotes chondrogenic differentiation of human mesenchymal stem cells. J Pineal Res. 2018;65:e12483. doi: 10.1111/jpi.12483.

41. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Procko DJ, Horwitz EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 2006, v. 8, n. 4, p. 315-317.

42. Zuk PA, Zhu M, Ashjian P. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell; 13: 4279–4295, 2002.

43. Caplan AI, Buder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. Trends Mol Med; 7: 259-64, 2001.

44. Thery C, Boussac M, Veron P, Ricciardi-Castagnoli P, Raposo G, Garin J, et al., Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles, J. Immunol. 166 (2001) 7309–7318.

45. Baharlooi H, Nouraei Z, Azimi M, Moghadasi AN, Tavassolifar MJ, Moradi B, Sahraian MA, Izad M. Umbilical cord mesenchymal stem cells as well as their released exosomes suppress proliferation of activated PBMCs in multiple sclerosis. Scand J Immunol. 2020 Dec 18:e13013. doi: 10.1111/sji.13013. Epub ahead of print. PMID: 33338274.

46. Mears R, Craven RA, Hanrahan S, Totty N, Upton C, Young SL, et al., Proteomic analysis of melanoma-derived exosomes by two-dimensional polyacrylamide gel electrophoresis and mass spectrometry, Proteomics 4 (2004) 4019–4031, https://doi.org/10.1002/pmic.200400876.

47. R. Gastpar, M. Gehrmann, M.A. Bausero, A. Asea, C. Gross, J.A. Schroeder, et al., Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells, Cancer Res. 65 (2005) 5238–5247, https://doi.org/10.1158/0008-5472.can-04-3804.

48. W. Xu, Z. Yang, N. Lu, From pathogenesis to clinical application: insights into exosomes as transfer vectors in cancer, J. Exp. Clin. Cancer Res. 35 (2016) 156, https://doi.org/10.1186/s13046-016-0429-5.

49. H. Valadi, K. Ekstrom, A. Bossios, M. Sjostrand, J.J. Lee, J.O. Lotvall, Exosomemediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (2007) 654–659, https://doi.org/10.1038/ncb1596.

50. B. Zhang, L. Shen, H. Shi, Z. Pan, L. Wu, Y. Yan, et al., Exosomes from Human Umbilical Cord Mesenchymal Stem Cells: Identification, Purification, and Biological Characteristics, 2016, p. 1929536, https://doi.org/10.1155/2016/1929536.

51. Zhuang G, Mao J, Yang G, Wang H. Influence of different incision designs on bone increment of guided bone regeneration (Bio-Gide collagen membrane +Bio-OSS bone powder) during the same period of maxillary anterior tooth implantation. Bioengineered. 2021 Dec;12(1):2155-2163. doi: 10.1080/21655979.2021.1932209. PMID: 34057023.

52. Mesimäki K, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R: Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg 2009, 38 : 201-209.

53. Zotarelli Filho IJ, Frascino LF, Greco OT, Araujo JDD, Bilaqui A, Kassis EN, Ardito RV and Bonilla-Rodriguez GO. Chitosan-collagen scaffolds can regulate the biological activities of adipose mesenchymal stem cells for tissue engineering. J Regen Med Tissue Eng. 2013; 2:12. http://dx.doi.org/10.7243/2050-1218-2-12.

54. Egido-Moreno S, Valls-Roca-Umbert J, Céspedes-Sánchez JM, López-López J, Velasco-Ortega E. Clinical Efficacy of Mesenchymal Stem Cells in Bone Regeneration in Oral Implantology. Systematic Review and Meta-Analysis. Int J Environ Res Public Health. 2021 Jan 21;18(3):894. doi: 10.3390/ijerph18030894.

55. Liang W, Han B, Hai Y, Sun D, Yin P. Mechanism of Action of Mesenchymal Stem Cell-Derived Exosomes in the Intervertebral Disc Degeneration Treatment and Bone Repair and Regeneration. Front Cell Dev Biol. 2022 Jan 14;9:833840. doi: 10.3389/fcell.2021.833840.

56. Shapira SN, Christofk HR. Metabolic Regulation of Tissue Stem Cells. Trends Cell Biol. 2020 Jul;30(7):566-576. doi: 10.1016/j.tcb.2020.04.004. Epub 2020 Apr 28. PMID: 32359707.

57. Lewis, B.A. et al. Human RNA polymerase II promoter recruitment in vitro is regulated by O-linked N-acetylglucosaminyltransferase (OGT). J. Biol. Chem. 2016, 291, 14056–14061.

58. Shanmugavadivu A, Balagangadharan K, Selvamurugan N. Angiogenic and osteogenic effects of flavonoids in bone regeneration. Biotechnol Bioeng. 2022 Sep;119(9):2313-2330. doi: 10.1002/bit.28162.

59. Nastri L, Moretti A, Migliaccio S, Paoletta M, Annunziata M, Liguori S, Toro G, Bianco M, Cecoro G, Guida L, Iolascon G. Do Dietary Supplements and Nutraceuticals Have Effects on Dental Implant Osseointegration? A Scoping Review. Nutrients. 2020 Jan 20;12(1):268. doi: 10.3390/nu12010268.